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COMMUNICATION

The Effect of a Mycotoxin Deactivation Product on Growth of Juvenile Rainbow Trout Fed Distillers Dried Grains

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Abstract

Distillers dried grains (DDG) with solubles (DDGS) is a product that has shown potential as a protein source for some fish species, but high inclusion rates of DDGS have not always been successfully achieved for Rainbow Trout *Oncorhynchus mykiss*. Our objective was to determine whether inclusion of a mycotoxin deactivation product (Biofix Plus) could improve the ability of high-protein DDG (HPDDG) to replace a portion of the fish meal in diets for Rainbow Trout. The 2 × 2 factorial feeding trial examined protein source (menhaden fish meal [MFM] or HPDDG) with or without Biofix Plus. A control diet (42% digestible protein, 20% crude lipid, 25% MFM) was compared to a test diet in which HPDDG replaced 12% of the total MFM on a digestible-protein basis (24% HPDDG inclusion). Diets were fed to juvenile Rainbow Trout (initial weight: mean ± SE = 30.5 ± 1.6 g) in four replicate tanks per treatment for 9 weeks in a 15°C recirculating system. At the conclusion of the feeding trial, we observed no negative effects of fish meal replacement on growth or feed conversion ratio; no benefit of Biofix Plus supplementation was observed. These data indicate that when Rainbow Trout diets containing a high-quality DDGS product are balanced for digestible protein, lysine, methionine, and threonine, dietary fish meal levels can be successfully reduced to 13% without compromising growth and without the need for mycotoxin deactivator inclusion.

With continued interest in renewable fuels, ethanol production increased more than 13-fold from 2000 to 2013; as a result, ethanol production now supplies more than 35.5 million tons of distillers dried grains (DDG) with solubles (DDGS) and other co-products (Lim and Yildirim-Aksoy 2008; RFA 2014). However, numerous reports have recently presented or cited data on detectable mycotoxins in DDGS, and concerns have been raised about the use of U.S. DDGS as animal feed (Garcia et al. 2008; Rodrigues 2008; Taylor-Pickard 2008; Wu and Munkvold 2008; Ogiso et al. 2013; Khatibi et al. 2014).

Mycotoxicosis was first recorded in Rainbow Trout *Oncorhynchus mykiss* in the early 1960s, when hatcheries experienced losses of 70–80% of their fish crop due to the presence of aflatoxin B1 in cottonseed meal that was used in prepared feed (Ashley and Halver 1963). Aflatoxins are the most studied cause of mycotoxicosis in fish, primarily due to the significant pathology observed in Rainbow Trout. Although less studied, other mycotoxins have also been reported to reduce the production efficiency and health of aquatic species during chronic low-level exposures. Hooft et al. (2011) reported that deoxynivalenol at 0.5 μg/g and higher levels decreased weight gain,

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feed intake, feed efficiency, and nutrient absorption in Rainbow Trout. Manning et al. (2003) reported a reduction in body weight gain for Channel Catfish *Ictalurus punctatus* that were fed diets with ochratoxin A at 1 μg/g. Reduced feed efficiency was also observed for dietary contamination with ochratoxin at 4 and 8 μg/g (Manning et al. 2003). Lumlertdacha et al. (1995) reported that weight gain in Channel Catfish was decreased at a fumonisin B1 level of 20 μg/g. Growth was similarly decreased in Nile Tilapia *Oreochromis niloticus* when fumonisin B1 was administered at dietary levels of 40 μg/g or higher (Tuan et al. 2003), whereas Common Carp *Cyprinus carpio* that received either 0.5- or 5-μg/g fumonisin B1 in feed exhibited a reduction in weight gain (Pepeljnjak et al. 2002). Only one preliminary study (Döll et al. 2011) has investigated the effects of zearalenone on fish growth performance. In that study, no adverse effects on weight gain of Atlantic Salmon *Salmo salar* were observed for dietary concentrations of zearalenone ranging from 60 to 770 ng/g.

The quality of raw maize grain and the processing conditions are known to contribute significantly to differences in nutrient densification and quality of resultant DDGS (Chevanan et al. 2007, 2008); therefore, these factors may partly explain the inconsistent results observed with DDGS inclusion in salmonid diets (Cheng and Hardy 2004; Barnes et al. 2012a, 2012b, 2012c; Øverland et al. 2013). Cheng and Hardy (2004) reported that DDGS could be included in Rainbow Trout diets at levels from 15% to 22.5%; furthermore, when lysine and methionine were supplemented, DDGS could effectively replace fish meal. However, Barnes et al. (2012a) reported that growth and feed conversion of juvenile Rainbow Trout were negatively impacted when the fish were fed diets containing 10% or 20% DDGS, even when supplemented with essential amino acids and phytase. Barnes et al. (2012b, 2012c) recommended similar levels of DDG incorporation (10% and 20%, respectively) in Rainbow Trout diets when using a further processed DDG product, high-protein DDG (HPDDG), which is produced by fractionating the maize and removing the nonfermentable fractions prior to ethanol production (Singh et al. 2005). More recently, Øverland et al. (2013) reported that up to 50% DDGS or 45% HPDDG could be included as replacement for plant proteins and could support Rainbow Trout growth equivalent to that of fish fed diets containing more traditional plant proteins as long as fish meal was included in the diet at approximately 20%.

The potential for mycotoxin contamination of the DDGS used in prior fish studies was largely uninvestigated but may explain some of the limitations previously observed in the utilization of DDGS as a fish meal replacement, especially in diets for Rainbow Trout. A notable exception is the paper by Øverland et al. (2013), who reported detectable levels of common mycotoxins in both of the DDGS products they examined. Øverland et al. (2013) cautioned that mycotoxin contamination is a potential risk factor that could limit the use of these maize co-products in the fish feed industry due to the fact that mycotoxin levels in DDGS can be concentrated 3.0 times (Wu and Munkvold 2008) to 3.5 times (Zhang and Caupert 2012) compared with levels in raw maize. Strategies that can mitigate potential risks are therefore needed to facilitate the use of DDGS products in mycotoxin-sensitive species. The purpose of the current study was to determine whether inclusion of a mycotoxin deactivation product could improve the ability of a DDGS product to replace a higher proportion of the fish meal in Rainbow Trout diets.

METHODS

Experimental approach.—To precisely formulate experimental diets for a growth trial with a DDGS product on a digestible-nutrient basis, it was necessary to first conduct an in vivo assessment of the digestibility of three different DDGS products. In the digestibility trial, we analyzed two commercially available DDGS products with moderate protein content (Valero DDGS [Valero Marketing and Supply, San Antonio, Texas] and Wentworth DDGS [Dakota Ethanol, Wentworth, South Dakota]), one commercially available HPDDG (produced by pre-fermentation separation of nonfermentable maize fractions), and menhaden fish meal (MFM). Samples of each DDGS product, MFM, wheat, maize protein concentrate, and soybean meal also were submitted to Romer Laboratories (Union, Missouri), for mycotoxin screening; specifically, aflatoxin, deoxynivalenol, fumonisin, zearalenone, and ochratoxin levels were determined for each ingredient. The HPDDG was found to contain deoxynivalenol (also known as vomitoxin) at 2.5 μg/g and zearalenone at 1,643 ng/g. All other ingredients tested had mycotoxin levels below detection limits. Subsequently, a feeding trial that utilized the HPDDG product as a substitute for a portion of the MFM protein in a practical-type Rainbow Trout feed tested whether a mycotoxin deactivation product could be used to improve Rainbow Trout production efficiency. All fish were handled and treated in accordance with guidelines approved by the U.S. Fish and Wildlife Service.

In vivo digestibility determinations.—The nutritional values of the three DDGS products (Valero DDGS, Wentworth DDGS, and HPDDG) were evaluated by determining the apparent digestibility coefficients (ADCs) of nutrients and energy and the apparent availability coefficients (AACs) of amino acids in compounded, extruded diets using the methods described by Cho et al. (1982) and Bureau et al. (1999). All diets were labeled using yttrium oxide as the inert marker. A complete reference diet (Table 1), which met or exceeded all known nutritional requirements for Rainbow Trout, was blended with the test ingredient (MFM, Valero DDGS, Wentworth DDGS, or HPDDG) in a 70:30 ratio (dry weight basis) to form each digestibility test diet. This reference diet has been used successfully in several recent digestibility trials with Rainbow Trout (Gaylord et al. 2008, 2010; Gaylord and Barrows 2009).

Digestibility diets were manufactured by cooking extrusion (DNDL-44; Buhler AG, Uzwil, Switzerland) with an

TABLE 1. Composition of the digestibility reference diet (g/kg dry weight) fed to Rainbow Trout for in vivo digestibility determinations; ARS = Agricultural Research Service.

Ingredient	Concentration (g/kg)
Wheat flour[a]	283
Squid meal[b]	250
Soy protein concentrate[c]	171
Fish oil[d]	134
Maize gluten meal[e]	83
Soybean meal[f]	43
Vitamin premix ARS[g]	10
Chromic oxide[h]	10
Choline chloride[h]	6
Taurine	5
Stay-C 35	2
Trace mineral premix[i]	1
Yttrium oxide[h]	1

[a]Archer Daniels Midland (Decatur, Illinois), 4 g protein/kg.
[b]Wilbur Ellis (Portland, Oregon), 800 g crude protein/kg.
[c]Solae Profine VP (St. Louis, Missouri), 693 g crude protein/kg.
[d]Omega Protein (Houston, Texas).
[e]Cargill Animal Nutrition (Minneapolis, Minnesota), 601.0 g protein/kg.
[f]Archer Daniels Midland, 480 g protein/kg.
[g]Contributed per kilogram of diet: vitamin A, 9,650 IU; vitamin D, 6,600 IU; vitamin E, 132 IU; vitamin K3, 1.1 g; thiamine mononitrate, 9.1 mg; riboflavin, 9.6 mg; pyridoxine hydrochloride, 13.7 mg; pantothenate DL-calcium, 46.5 mg; cyanocobalamin, 0.03 mg; nicotinic acid, 21.8 mg; biotin, 0.34 mg; folic acid, 2.5 mg; and inositol, 600 mg.
[h]Sigma-Aldrich (St. Louis, Missouri).
[i]Contributed per kilogram of diet: zinc, 40 mg; manganese, 13 mg; iodine, 5 mg; and copper, 9 mg.

approximate 18-s residence time at an average barrel temperature of 127°C. Pressure at the die head varied from 1.8 to 3.1 MPa (260–450 lb/in^2) depending on the diet. The die plate was cooled to an average temperature of 60°C. Pellets of 3–4 mm (diameter and length) were produced and dried in a pulse-bed drier (Buhler AG) to less than 10% moisture, followed by a 20-min cooling period at ambient temperature. All diets were top-coated with fish oil by using a vacuum coater (A&J Mixing, Oakville, Ontario).

The digestibility diets were fed to 30 fish in each 500-L tank (individual weight of each fish = 500–700 g). A diet was randomly assigned to each tank, and each diet was fed to three different tanks. Fish were fed by hand twice daily to satiation. Water temperature was maintained at 15°C, and lighting was maintained on a cycle of 13 h light : 11 h dark. Fecal samples were obtained by manual stripping at 16–18 h postfeeding. Manual stripping of all fish in a given tank was accomplished by netting and anesthetizing the fish, followed by gently drying and then applying pressure to the lower abdominal region. First, urine was expressed into a waste container, and then fecal matter was expressed into a plastic weighing pan. Fecal samples from each tank were freeze dried and stored at −20°C until chemical analyses were performed.

The ADC and AAC of each nutrient in the test diet and the individual ingredients were calculated according to the following equations (Kleiber 1961; Forster 1999):

$$\mathrm{ADCN}_{diet} = 100 \times \frac{(\%\ \text{marker in diet}) \times (\%\ \text{nutrient in feces})}{(\%\ \text{marker in feces}) \times (\%\ \text{nutrient in diet})}$$

$$\mathrm{ADCN}_{ingredient} = \{[(a+b)\cdot \mathrm{ADCN}_t] - (a \cdot \mathrm{ADCN}_r)\} \cdot b^{-1},$$

where

$\mathrm{ADCN}_{ingredient}$ = ADC of the nutrient in the test ingredient;
ADCN_t = ADC of the nutrient in the test diet;
ADCN_r = ADC of the nutrient in the reference diet;
$a = (1 - p) \times$ (nutrient content of the reference diet);
$b = p \times$ (nutrient content of the test ingredient); and
p = proportion of the test ingredient in the test diet.

Growth trial.—A 2 × 2 factorial feeding trial that examined protein source (MFM or HPDDG) and Biofix Plus supplementation (with or without) was conducted (Table 2). The control diet was compared with a test diet in which HPDDG replaced 12% of the MFM on a digestible-protein basis (23% dietary inclusion of HPDDG). Biofix Plus is a mycotoxin deactivation product that contains yeast cell wall fractions, natural microbials, enzymatic activity, and diatomaceous earth; this product was chosen because it is marketed to be effective against deoxynivalenol. The manufacturer's recommended inclusion level for Biofix Plus was used in the test diet.

All diets that were administered during the feeding trial met or exceeded National Research Council requirements (NRC 1993, 2011) for Rainbow Trout and were formulated to contain 42% digestible protein and 20% crude lipid on a dry matter basis. Diets were balanced for available lysine (3.82%), methionine (1.30%), and threonine (2.14%) as well as total phosphorus (Table 2) based on digestible nutrient data from the digestibility trial. Diets used in the feeding trial were manufactured by cooking extrusion with the same equipment and processing conditions as in the digestibility trial. Biofix Plus was supplemented (0.1%) to diets via vacuum-assisted top coating in the dietary oil portion. However, because a large quantity of fines was observed in diets containing HPDDG, we found it necessary to assess pellet quality by using a Holmen NHP100 portable pellet durability tester (TekPro, Norfolk, UK). Briefly, approximately 50 g of pellets were loaded into the test chamber, which then cascaded the pellets in an air stream for 60 s, causing them to collide with each other and perforated surfaces within the test chamber. After the test cycle, sample pellets were ejected from the tester for manual weighing. The pellet durability index was calculated as the difference between pellet weights measured before and after the test, expressed as a percentage of initial weight. Pellet durability indices were determined on triplicate pellet samples from each diet. Based on these results, diets were ground and re-pelleted via cold extrusion (i.e., no heat was used) to ensure that pellet quality was equivalent among test diets.

TABLE 2. Ingredients and composition of test diets fed to juvenile Rainbow Trout in the growth trial; diets contained menhaden fish meal (MFM) or high-protein distillers dried grains (HPDDG) with or without Biofix Plus (a mycotoxin deactivator).

	Diet			
Ingredient (g/kg) or component	MFM	MFM with Biofix Plus	HPDDG	HPDDG with Biofix Plus
HPDDG[a]	0.0	0.0	235	235
MFM, Special Select[b]	247.5	247.5	133	133
Biofix Plus[c]	0	1	0	1
Maize protein concentrate[d]	50	50	50	50
Blood meal[e]	30	30	30	30
Soybean meal (solvent-extracted and de-hulled)[b]	150	150	150	150
Poultry by-product meal (pet food grade)[b]	143	143	143	143
Wheat flour[f]	164.1	163.1	27.8	26.8
Menhaden fish oil[c]	146	146	143	143
Lecithin	10	10	10	10
Stay-C 35	2	2	2	2
Vitamin premix[g]	10	10	10	10
Trace mineral premix[h]	1	1	1	1
Sodium chloride	2.8	2.8	2.8	2.8
Magnesium oxide	0.6	0.6	0.6	0.6
Potassium chloride	5.6	5.6	5.6	5.6
Dicalcium phosphate	0.0	0.0	12.5	12.5
Choline chloride	10	10	10	10
DL-methionine	3.8	3.8	5.0	5.0
Lysine hydrochloride	16	16	20	20
Threonine	1.6	1.6	2.5	2.5
Taurine	5	5	5	5
Yttrium oxide	1	1	1	1
Analyzed composition[i]				
Crude protein (g/kg)	489	492	486	486
Crude lipid (g/kg)	198	208	201	211
Gross energy (MJ/kg)	22.4	22.4	22.9	22.9
Moisture (g/kg)	37	43	48	45
Post-extrusion PDI (%)[j]	27	28	44	46
Post-re-pelleting PDI (%)[k]	33	32	34	33

[a]POET Nutrition (Sioux Falls, South Dakota).
[b]Nelson and Sons (Murray, Utah).
[c]Biomin USA (San Antonio, Texas).
[d]Cargill Animal Nutrition (Minneapolis, Minnesota), 601.0 g protein/kg.
[e]Gavilon (Omaha, Nebraska).
[f]MGP Ingredients (Atchison, Kansas).
[g]Contributed per kilogram of diet: vitamin A (as retinol palmitate), 30,000 IU; vitamin D_3, 2,160 IU; vitamin E (as DL-alpha-tocopheryl-acetate), 1,590 IU; niacin, 990 mg; calcium pantothenate, 480 mg; riboflavin, 240 mg; thiamine mononitrate, 150 mg; pyridoxine hydrochloride, 135 mg; menadione sodium bisulfate, 75 mg; folacin, 39 mg; biotin, 3 mg; and vitamin B_{12}, 90 μg.
[h]Contributed per kilogram of diet: zinc, 37 mg; manganese, 10 mg; iodine, 5 mg; copper, 3 mg; and selenium, 0.4 mg.
[i]Means of two replicate samples per diet on a dry matter basis.
[j]Post-extrusion PDI = pellet durability index determined immediately after cooking extrusion.
[k]Post-re-pelleting PDI = pellet durability index determined after re-pelleting by cold extrusion.

All four diets (Table 2) were fed to juvenile Rainbow Trout (initial weight: mean ± SD = 30.5 ± 1.6 g) in four replicate tanks per treatment for 9 weeks in a 15°C recirculating system. Bulk fish weight and feed intake were recorded every 3 weeks. Five fish from the original population were sacrificed for determination of initial whole-body proximate composition. At the conclusion of the trial, three fish from each tank were sampled for whole-body composition, and three additional fish were used for determination of hepatosomatic index (HSI), muscle ratio (MR), and viscerosomatic index (VSI). The following

formulae were used:

$$\text{Weight gain (\% increase)} = \frac{(\text{final weight, g}) - (\text{initial weight, g})}{(\text{initial weight, g})} \times 100;$$

$$\text{Feed conversion ratio (FCR; feed:gain)} = \frac{(\text{wet diet fed, g})}{(\text{wet weight gained, g})};$$

$$\text{Feed intake (\% body weight [BW]/d)} = (\text{wet diet fed, g}) \times 100 \frac{\left[(\text{initial weight, g}) - (\text{final weight, g})\right]/2}{\text{number of days}};$$

$$\text{HSI (\%)} = \frac{(\text{liver weight, g})}{(\text{whole-body weight, g})} \times 100;$$

$$\text{VSI (\%)} = \frac{(\text{viscera weight, g})}{(\text{whole-body weight, g})} \times 100; \text{ and}$$

$$\text{MR (\%)} = \frac{(\text{fillet weight, g}) \times 2}{(\text{whole-body weight, g})} \times 100.$$

Analytical methods.—Dry matter analyses of ingredients, diets, and feces were performed according to standard methods (AOAC International 1995). Yttrium and phosphorus were determined in diets and feces by using inductively coupled plasma atomic absorption spectrophotometry after nitric acid digestion (Anderson 1996). Briefly, duplicate samples of diets (0.5 g) and feces (1 g) were digested in 10 mL of concentrated (70%) nitric acid at 95 C for 7 h. After digestion, samples were vacuum filtered through a 0.45-μm filter and were diluted to 50 mL with deionized water to the appropriate concentration (14% HNO_3 for micro-mineral analysis; 4% HNO_3 for macro-mineral analysis). Samples were analyzed by using an Optima 5300 DV trace mineral analyzer (Perkin-Elmer, San Diego, California) and were quantitated by simultaneous analysis of defined standards (High-Purity Standards, Charleston, South Carolina). Crude protein (N × 6.25) in ingredients, diets, and feces was determined by the Dumas method (AOAC International 1995) on a LECO TruSpec-N nitrogen determinator (LECO, St. Joseph, Michigan). Total energy was determined by isoperibol bomb calorimetry (Parr 6300; Parr Instrument, Moline, Illinois). Crude lipid was determined by petroleum ether extraction using an Ankom XT10 (Ankom Technologies, Macedon, New York). Amino acids in the diets, ingredients, and feces were quantified after acid hydrolysis by utilizing a Beckman 7300 amino acid analyzer and postcolumn derivatization with ninhydrin (AAA Laboratory, Mercer Island, Washington).

Statistical analyses.—Factorial ANOVA was performed by using the general linear models procedure (PROC GLM) in SAS version 9.1 (SAS Institute, Cary, North Carolina). When significant interactions were observed, Tukey's mean separation test (Tukey 1953) was used. Treatment effects were considered significant at *P*-values less than 0.05.

TABLE 3. Proximate composition and amino acid composition of high-protein distillers dried grains (HPDDG), distillers dried grains with solubles (DDGS), and menhaden fish meal (MFM). Values are means of duplicate analyses and are reported on a dry matter basis (g/kg of sample, unless otherwise noted).

Component or amino acid	POET HPDDG	Valero DDGS	Wentworth DDGS	MFM
Dry matter	935	858	843	930
Crude protein	408	305	325	676
Crude lipid	54	105	129	80
Gross energy (MJ/kg)	22.3	22.8	23.9	19.6
Phosphorus	4	9	10	33
Amino acids (sum)	418	278	306	651
Alanine	33	22	24	46
Arginine	17	13	16	48
Aspartic acid	29	20	23	66
Glutamic acid	77	45	49	96
Glycine	14	12	13	52
Histidine	10	7	8	15
Isoleucine	16	10	12	28
Leucine	58	36	39	52
Lysine	10	8	9	45
Methionine	6	4	4	14
Phenylalanine	23	15	17	29
Proline	39	25	27	35
Serine	25	17	19	32
Threonine	19	14	16	33
Tyrosine	20	13	14	23
Valine	22	16	17	37

RESULTS

The proximate composition and amino acid levels of the DDGS products and MFM are shown in Table 3. Protein ADCs were 0.85 for HPDDG, 0.88 for Valero DDGS, and 0.81 for Wentworth DDGS, compared to 0.91 for MFM (Table 4). Lipid ADCs averaged 0.80 for the DDGS products and 0.89 for MFM. Energy ADCs ranged from 0.54 to 0.59 for the DDGS products, whereas the energy ADC for MFM was 0.99 (Table 4). Lysine AAC ranged from 0.68 to 0.79 for the DDGS products and was 0.97 for MFM. Methionine AAC was also variable in DDGS products, ranging from 0.75 to 0.99; the methionine AAC for MFM was 0.95.

Macronutrient composition of diets in the growth trial generally reflected formulation targets (Table 2). Pellet durability index values for pellets produced by cooking extrusion were significantly affected by DGGS inclusion such that pellet losses increased from 27.5% for the MFM diets to 45% for the HPDDG diets (Table 2), resulting in considerable fines. These

TABLE 4. Apparent digestibility coefficients (ADCs) and apparent availability coefficients (AACs) for high-protein distillers dried grains (HPDDG), distillers dried grains with solubles (DDGS), and menhaden fish meal (MFM) fed to Rainbow Trout. Values are means for three replicate tanks (30 fish/tank).

Component or amino acid	POET HPDDG	Valero DDGS	Wentworth DDGS	MFM
		ADCs		
Dry matter	0.52	0.50	0.40	0.79
Crude protein	0.85	0.88	0.81	0.91
Crude lipid	0.79	0.79	0.83	0.89
Gross energy	0.59	0.59	0.54	0.99
Phosphorus	0.80	0.91	0.78	0.45
		AACs		
Alanine	0.84	0.92	0.82	0.91
Arginine	0.89	0.99	0.90	0.95
Aspartic acid	0.70	0.91	0.69	0.95
Glutamic acid	0.83	0.95	0.83	0.96
Glycine	0.56	0.81	0.57	0.79
Histidine	0.82	0.90	0.79	0.97
Isoleucine	0.78	0.89	0.76	0.99
Leucine	0.90	0.97	0.86	1.0
Lysine	0.69	0.79	0.68	0.97
Methionine	0.75	0.99	0.85	0.95
Phenylalanine	0.67	0.82	0.68	0.93
Proline	0.83	0.92	0.80	0.83
Serine	0.73	0.88	0.77	0.94
Threonine	0.67	0.78	0.72	0.96
Tyrosine	0.88	0.95	0.88	0.98
Valine	0.77	0.86	0.76	0.96

findings parallel those of Chevanan et al. (2007, 2008), who also found challenges in producing viable extrudates when using DDGS. After grinding and re-pelleting, the pellet durability index ranged from 32% to 34% for all diets (Table 2).

Weight gain, FCR, and feed intake were not significantly affected by dietary protein source, Biofix Plus supplementation, or the interaction of protein source × Biofix Plus supplementation (Table 5). Weight gain (% increase) ranged from 435% to 484% and averaged 443% for Rainbow Trout that were fed diets containing HPDDG; a weight gain of 445% was observed for fish that received diets containing MFM. The FCR averaged 0.95 for fish that were fed diets with HPDDG but averaged 0.80 for fish that were given diets with MFM. Feed intake averaged 2.6% BW/d for Rainbow Trout that were given diets with HPDDG compared to 2.4% BW/d for fish that were fed MFM diets. Body condition indices in the growth trial were similarly nonresponsive to diet (Table 5). The VSI ranged from 9.9 to 11.7, MR ranged from 49.2 to 52.5, and HSI ranged from 0.9 to 1.0. Whole-body proximate composition was not significantly altered by dietary protein source, Biofix Plus supplementation, or their interaction (Table 6). Ranges of proximate components were as follows: 67.8–69.1% for moisture; 12.7–14.2% for lipid; and 15.9–16.2% for protein. Gross energy levels of Rainbow Trout ranged from 8.7 to 9.1 MJ/kg.

DISCUSSION

The protein ADCs for Rainbow Trout when fed the three DDGS products evaluated in the present study were higher than the value of 0.72 reported by Smith et al. (1980) for distillers dried solubles and were slightly lower than the value of 0.90 reported by Cheng and Hardy (2004) in a study of DDGS. The high values of protein ADC in our study were supported by the similarly high amino acid ADCs. Utilizing these available

TABLE 5. Growth performance variables and condition indices for Rainbow Trout that were fed diets containing menhaden fish meal (MFM) or high-protein distillers dried grains (HPDDG) with or without Biofix Plus (FCR = feed conversion ratio; BW = body weight; VSI = viscerosomatic index; MR = muscle ratio; HSI = hepatosomatic index). Results of statistical analyses (*P*-values) are also shown. See Methods for equations used to calculate the performance variables and condition indices.

	Growth performance[a]			Condition indices[b]		
Diet or effect	Weight gain (% increase)	FCR(g feed/g gain)	Feed intake (% BW/d)	VSI (%)	MR (%)	HSI (%)
MFM	484	0.9	2.3	10.6	51.2	0.9
MFM with Biofix Plus	408	1.0	2.5	9.9	52.5	0.9
HPDDG	451	1.0	2.6	10.3	49.2	0.9
HPDDG with Biofix Plus	435	1.0	2.5	11.7	50.9	1.0
Pooled SE	22	0.05	0.11	0.4	1.0	0.06
P-values						
Protein source	0.9130	0.4881	0.3435	0.1137	0.0797	0.8139
Biofix Plus	0.0561	0.3091	0.4593	0.3767	0.1413	0.3358
Protein source × Biofix Plus	0.1882	0.2906	0.3540	0.0205	0.8403	0.3591

[a]Means of four replicate tanks (15 fish/tank).
[b]Means for 3 fish/tank (4 replicate tanks/diet treatment).

TABLE 6. Proximate composition of Rainbow Trout that were fed diets containing menhaden fish meal (MFM) or high-protein distillers dried grains (HPDDG) with or without Biofix Plus. Results of statistical analyses (*P*-values) are also shown. Values represent the means for 3 fish/tank (4 replicate tanks/diet treatment).

Diet or effect	Moisture (g/kg)	Crude lipid (g/kg)	Crude protein (g/kg)	Gross energy (MJ/kg)
MFM	691	127	162	8.7
MFM with Biofix Plus	681	142	159	9.1
HPDDG	681	136	161	8.7
HPDDG with Biofix Plus	678	138	161	9.1
Pooled SE	7	6	2	0.5
P-values				
Protein	0.4311	0.6418	0.8987	0.9427
Biofix Plus	0.4224	0.1945	0.5554	0.0835
Protein source × Biofix Plus	0.6587	0.3326	0.4948	0.9755

amino acid values in the present study allowed for a reduction in fish meal level from approximately 25% to 13% of the diet and the inclusion of HPDDG at 23% of the diet but without compromising Rainbow Trout growth or production efficiency as long as lysine, methionine, and threonine were supplemented. Barnes et al. (2012a) reported a reduction in growth rate and an increase in FCR when 10% DDGS was included and replaced fish meal, maize gluten meal, and wheat in diets for Rainbow Trout, even when the diets were supplemented with essential amino acids and phytase. Barnes et al. (2012b, 2012c) recommended similar levels of HPDDG incorporation (10% and 20%, respectively) in Rainbow Trout diets. More recently, Øverland et al. (2013) included much higher amounts of DDGS (50%) and HPDDG (45%) without negative effects on Rainbow Trout when fish meal was included at 18% and when equal amounts of lysine and methionine were supplemented to all diets. Our results further the work of Øverland et al. (2013) by successfully reducing the fish meal level to 13% via HPDDG replacement without compromising growth.

Although limited research has investigated the potential importance or confounding effects of low-level mycotoxin contamination in alternative ingredient evaluations, the use of mycotoxin deactivation products in contaminated fish feeds has previously been shown to increase growth performance (Abdelaziz et al. 2010; Agouz and Anwer 2011). Thus, the present study was designed to address this potential limitation of DDGS inclusion in aquafeeds. However, our results do not agree with observations reported by Abdelaziz et al. (2010) and Agouz and Anwer (2011), who found substantial increases in fish performance when mycotoxin binder products were included in mycotoxin-contaminated feeds. Possible reasons for these differing results include differences in (1) the types and levels of mycotoxins examined and (2) the mycotoxin deactivators utilized. In our study, the mycotoxins present in the HPDDG sample were deoxynivalenol and zearalenone, whereas Abdelaziz et al. (2010) and Agouz and Anwer (2011) evaluated fish feed that was contaminated with aflatoxin and ochratoxin. Additionally, the calculated mycotoxin levels based on the 24% HPDDG inclusion level in the current study were 0.6 μg/g for deoxynivalenol and 410 ng/g for zearalenone. Although deoxynivalenol at 0.6 μg/g is above the 0.5-μg/g level that was shown to be detrimental (Hooft et al. 2011), Øverland et al. (2013) did not observe adverse effects of similar levels (0.55 μg/g) on fish health as measured by gastrointestinal health and plasma metabolites. Our results and those of Øverland et al. (2013) seem to suggest that these levels and combinations of mycotoxins in DDGS products do not negatively impact the growth performance of Rainbow Trout. Alternatively, because mycotoxin contamination is often not homogeneous throughout a batch of a given ingredient, it is possible that the actual mycotoxin levels in the finished feeds are different from those calculated based on the analyzed concentrations in the ingredient.

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